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Sickle Cell Disorder, β-Globin Gene Cluster Haplotypes and α-Thalassemia in Neonates and Adults from Guadeloupe

L. Kéclard,[1]* M. Romana,[1] E. Lavocat,[2] C. Saint-Martin,[2] C. Berchel,[1] and G. Mérault[1]

[1]INSERM U.359, CHRU Pointe-à-Pitre, Pointe-à-Pitre, Guadeloupe, France
[2]Centre Intégré de la Drépanocytose, CHU Pointe-à-Pitre, Pointe-à-Pitre, Guadeloupe, France

We have studied haplotype of β^S chromosome and α-globin gene status in 534 patients (255 adults and 279 children of whom 159 neonates) from Guadeloupe with various sickle cell-related conditions, namely SS (n = 298), SC (n = 170), S-β-thal (n = 56), and other rare forms (n = 10). Haplotype data on β^S chromosomes confirm our previous observation that Benin type is the most prevalent (75%) β^S chromosome in Guadeloupe, in disagreement with the historical records. Comparison of the frequency of distribution of various β^S haplotypes between neonates and adults on the one hand and between SS and SC cases on the other shows that the current β^S haplotype distribution in this island is not distorted by haplotype-related differential survival. We also show that the frequency of α-thalassemia (−3.7 kb) in Guadeloupe is one of the highest recorded in this region involved in Atlantic slave trade and also failed to reveal any age-dependent increase in frequency. We conclude that the African component of Guadeloupe is distinct from that of Brazil and Cuba but is close to that of Jamaica. Am. J. Hematol. 55:24–27, 1997.

Key words: sickle cell disease; β-globin haplotype; α-thalassemia

INTRODUCTION

The β-globin gene cluster haplotypes have been used as genetic markers in examining the origin and spread of β-globin gene mutations, especially β^S gene, and also in studying the epistatic effects of linked genes in modulating the clinical expression of sickle cell disease [1–5]. Furthermore, by assessing the age-dependent variation in the frequency of β^S haplotypes, differential survival of these haplotypes in a given population could be estimated and, by assumption, the haplotype-related severity. In a previous report, studying 154 pediatric sickle cell disease patients from Guadeloupe, we observed that 74% of β^S haplotypes were of ''Benin'' type, while the ''Bantu'' and ''Senegal'' β^S haplotypes represented, respectively, 11% and 8% of β^S chromosomes [6]. These data were in total disagreement with the historical records, which favor an almost equal representation of Benin and Bantu β^S haplotypes, given the reported African ports of origin of present-day Guadeloupeans [7]. Since the studied population consisted essentially of children, Nagel [8] argued that the over representation of Benin and Senegal β^S haplotypes in this population could be due to their better survival, as compared to those with Bantu β^S haplotypes. Such an age-dependent distortion in the frequency of β^S haplotypes had already been noted in Cuba [9]. In order to clarify this issue we have now extended our studies, which include both neonate and adult patients with sickle cell disease (SCD). We have also explored whether the frequency of α-thalassemia in this population exhibit age dependency.

MATERIALS AND METHODS

Materials/Subjects

The study was performed on 534 subjects from the Centre Intégré de la Drépanocytose de Guadeloupe

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*Correspondence to: L. Kéclard, Unité de Recherche sur la Drépanocytose, INSERM U359, CHU Pointe-à-Pitre, BP 465, Pointe-à-Pitre, Guadeloupe FWI, France.

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TABLE I. Distribution of β^S Haplotypes Among Patients With Sickle Cell Disease

	Global[a]		Adult		Neonatal	
β^S haplotype	N	%	N	%	N	%
Benin (≠19)	622	74.8	283	76.3	174	75.7
Bantu (≠20)	92	11.1	36	9.7	23	10.0
Senegal (≠3)	51	6.1	20	5.4	10	4.3
Cameroon (≠17)	19	2.3	12	3.2	5	2.2
India (≠31)	6	0.7	5	1.3	1	0.4
Others	42	5.0	15	4.1	17	7.4
Total	832		371		230	

[a]Global sample includes non-neonatal pediatric samples.

TABLE II. Distribution of β^S Haplotypes by Age Group in SS and SC Patients

	SS adults		SS neonatal		SC adults		SC neonatal	
β^S haplotype	N	%	N	%	N	%	N	%
Benin (≠19)	222	77.1	132	76.7	61	73.5	42	72.4
Bantu (≠20)	28	9.7	15	8.7	8	9.7	8	13.8
Senegal (≠3)	15	5.2	8	4.7	5	6.0	2	3.4
Cameroon (≠17)	7	2.4	3	1.8	5	6.0	2	3.4
India (≠31)	5	1.8	0	0	0	0	1	1.8
Others	11	3.8	14	8.1	4	4.8	3	5.2
Total	288		172		83		58	

(Sickle Cell Center): 255 adults and 279 children, of whom 159 were neonates identified in a screening program. The adult sample consisted of 114 males and 141 females (mean age: 31.24 ± 12.42 years). The mean age of the pediatric sample (147 males and 132 females) was 8.95 ± 4.74 years. Of all these subjects, both pediatric and adult, 298 were SS, 170 were SC, 56 were S-β-thalassemia (β-thal), 5 with Hb S-hereditary persistence of fetal hemoglobin, and 5 were compound heterozygotes for sickle cell mutation and a structural variant (Hb E, Hb Lepore, Hb D Iran, Hb D Korlebu, or Hb D Punjab). All patients provided consent to participate in this study and parental consent was obtained for the children.

Identification of hemoglobin (Hb) type was made by isoelectrofocusing (IEF), citrate agar electrophoresis, and cation-exchange high-performance liquid chromatography (HPLC). Diagnosis of β-thalassemia was confirmed by mutation identification using polymerase chain reaction (PCR) and allele-specific oligonucleotide dot-blot hybridization (ASO) [10].

DNA was prepared from peripheral blood leukocytes as previously described [11]. Haplotype analysis was performed by a PCR-based approach [12]. The number of α-globin genes was determined by a PCR method [13]. Parallel Southern blot analysis of 50 randomly taken samples for α-globin genes status confirmed the PCR data.

Statistical Data

Proportions were compared with the χ^2 test, and $P < 0.05$ was considered statistically significant. Gene frequencies were determined by the counting method and submitted to χ^2 test.

RESULTS

The distribution pattern of β^S chromosomal haplotypes determined for 534 patients (832 β^S chromosomes), each at least with one β^S chromosome, is shown in Table I. Among the β^S haplotypes, Benin haplotype (Ben) was largely predominant (74.8%), followed by Bantu (CAR) (11.1%), Senegal (Sen) (6.1%), Cameroon (Cam) (2.3%), Arab-Indian (Ind) (0.7%), and all the remaining (5%) being atypical haplotypes. No statistically significant difference was noted in the haplotype distribution between adult (73.6% Ben, 9.7% CAR, 5.4% Sen, 3.2% Cam, 1.3% Ind, 4.1% atypical haplotypes) and neonatal groups (75.7% Ben, 10% CAR, 4.3% Sen, 2.2% Cam, 0.4% Ind, 7.4% atypical haplotypes) ($0.3 > P > 0.5$).

The frequency distribution of the β^S haplotypes was compared between SS (n = 230) and SC (n = 141) patients on the one hand and according to the age group on the other: adult (n = 227) and neonatal (n = 144). The results are summarized in Table II. The four groups exhibited similar pattern of distribution and the difference was statistically not significant.

The α-gene status was studied in 499 patients with SCD, of which 269 were SS, 138 SC patients, all the remaining being other forms of SCD. About 33% of patients had a deletional α-thal (−3.7 kb) with a gene frequency of 0.17 and 10 patients with triplicated α-globin gene arrangement. When the analysis of α-globin gene status was performed according to the age group, adult (n = 235) versus neonates (n = 119), no difference in distribution of different α-globin genotypes was noted, except for an intriguing (contrary to expectations) decrease in the frequency of homozygous form of α-thalassemia in adult patients (Table III).

Similar analysis by comparing SS versus SC patients failed to reveal any difference in the frequency distribution of different α-globin genotypes (Table IV). Overall, similar frequencies of α-thal gene (−3.7 kb) were observed for these four groups (0.17 for whole sample, 0.19 for SS, 0.17 for SC, 0.17 for adult, 0.20 for neonatal patients) and the differences were not statistically significant ($0.4 < P < 0.9$).

DISCUSSION

Earlier we reported that Benin β^S haplotype was the most prevalent haplotype in Guadeloupean pediatric patients with sickle cell disease amounting to three-fourths

TABLE III. α-Globin Gene Status by Age Group in Patients With Sickle Cell Disease

	All patients		Adult patients		Neonatal patients	
α-Gene status	N	%	N	%	N	%
αα/αα	330	66.1	154	65.6	73	61.3
−α/αα	153	30.7	76	32.3	37	31.1
−α/α−	6	1.2	1	0.4	4	3.4
ααα/αα	7	1.4	4	1.7	2	1.7
ααα/α−	3	0.6	0	0	3	2.5
Total	499 (f = 0.17)		235 (f = 0.17)		119 (f = 0.20)	

TABLE IV. α-Globin Gene Status in SS and SC Patients

	SS patients		SC patients	
α-Gene status	N	%	N	%
αα/αα	167	62.1	91	65.9
−α/αα	91	33.8	42	30.4
−α/α−	4	1.5	2	1.5
ααα/αα	4	1.5	3	2.2
ααα/α−	3	1.1	0	0
Total	269 (f = 0.19)		138 (f = 0.17)	

of the total β^S haplotypes followed, at a considerable distance, by Bantu (11%), Senegal (6%), and various other types (8%) [6]. These data were unexpected, given the historical records, which predicted an almost equal representation of Bantu and Benin β^S haplotypes with much less (3%) Senegal β^S haplotype in this part of the Caribbean islands [7]. Nagel [8] argued that the design of the study (study of only pediatric patients with sickle cell disease) was inappropriate to gain insight into the ports of origin of the African component to the present-day population of Guadeloupe, since the data are susceptible to distortions in haplotype frequencies by differential haplotype-related survival among SCD patients.

From the present study of a much larger cohort, which included adult SCD cases as well as additional children derived from neonatal screening, we confirm our previous findings: the Benin β^S haplotype is the most prevalent form in Guadeloupe. In addition, we show that the differential haplotype-related survival could not account for the poor representation of Bantu β^S haplotype in this island, since the frequencies of different β^S haplotypes were quite similar between neonate and adult SCD cases. Hence, the situation in Guadeloupe is quite different from that in Brazil [14] and Cuba [9], where Bantu and Benin β^S haplotypes are equal in frequency. The loss of Bantu β^S haplotype and gain of Senegal β^S haplotype in adult SCDs observed in Cuba must be characteristic of regions in which Bantu β^S haplotype (associated with the most severe phenotype) is significantly represented. In other words, haplotype-related differential survival will be manifest only in populations in which chances of having sickle cell anemia patients homozygous for Bantu β^S haplotype are higher (45% Bantu β^S haplotype in Cuba versus 11% in Guadeloupe). Indeed, in Guadeloupe we have so far identified only one case homozygous for Senegal β^S haplotype and four cases homozygous for Bantu β^S haplotype. In this context, it is of interest to note that in the regions that were under the influence of British and French slave trade, Benin β^S haplotype emerges as the prevalent present-day haplotype (United States, 50–60%, Jamaica 70%, and Guadeloupe 75%), while in those originally under Spanish and Portuguese influence, the presence of Bantu β^S haplotype is significant (Bresil 62%, Cuba 39%, and Surinam 30%).

TABLE V. α-Thalassemia Frequency in SS Patients from Cuba and Guadeloupe According to Age Group*

	Cuba		Guadeloupe	
Age (yr)	Frequency	No.	Frequency	No.
0–20	0.14	74	0.18	169
21–50	0.19	144	0.2	110
>50	0.34	16	0.2	10
Total		234		289

*In the Cuban groups, statistically significant difference was found between the age decade 0–20 years and 21–50 years ($P < 0.05$) and between the age decade 0–20 years and >50 years ($P < 0.001$). In the Guadeloupean groups, the differences were not statistically significant ($0.4 < P < 0.9$).

In Guadeloupe, deletional α-thalassemia (−3.7 kb) was found in about 37% of subjects with SCD, one of the highest figures so far reported for a region involved in Atlantic slave trade [15]. In addition, there is no statistically significant difference in α-thalassemia frequency between adults and neonates on the one hand and normal and sickle cell subjects on the other. However, this percentage is more likely an underestimation because other deletion and nondeletion forms had not been explored. In addition, no age-dependent difference in α-globin gene status was observed, suggesting that α-thalassemia trait did not contribute to differential survival among these patients. A recent study of SCD patients from Cuba shows that there is an age-dependent increase in frequency of α-thalassemia in a population divided by age decades [16], but we failed to observe any such tendency (Table V) when the data were projected in the same manner.

It is nevertheless important to point out that we have studied the effect of β-globin haplotype and α-thalassemia (−3.7 kb) on differential survival according to age group. These studies do not address the issue of the modulating effect of these two genetic variables on the clinical expression of sickle cell disease.

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